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Abstract

1) Normal karyotype of Donryu strain rat was determined according to the classification of KURITA et al. (8). Namely, the number of chromosomes was 42 in diploid cells, and chromosomes were divided into 3 groups (A, B and C) according to the position of centromere. A.group was consisted of 7 pairs of metacentric chromosomes, B-group 4 pairs of sub· meta.subtelocentrics and C.group 10 pairs of telocentrics and Y. 2) Among all chromosome pairs a pair of the longest telocentric chromosomes (C.l), 4 pairs of all the B.group, and the Y chromosome were recognizable. 3) The presence of polymorphism was demonstrated in the smallest submetacentric chromosomes (BA),: namely, (I) a homologous submeta. centric pair, (II) a homologous subtelocentric pair and (III) a heteromor. phic submeta and subtelocentric pair which seemed to be a hybrid from (I) and (II). To distinguish the polymorphism in their genotype from phenotype was impossible. 4) Animals with type III B·4 chromosomes were produced by type I and type II animals. 5) By checking the chromosomes of the inbred Donryu strain rats maintained over 40 generations by brother.sister mating at Nihon Rat Co., polymorphism in BA chromosomes was also recognized.

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AUTOSOMAL POLYMORPHISM IN DONRYU STRAIN RATS

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Chromosomal polymorphism in laboratory stocks and wild population of rats (*Rattus norvegicus*) has been reported by some investigators. Sex chromosome polymorphism of the animals has been first found by HUNGERFORD and NOWELL (1). In autosomes, polymorphism in No. 3 chromosomes and in the smallest subtelocentrics was observed by YOSIDA and AMANO (2), and BIANCHI and MOLINA (3), respectively.

In Donryu strain rats we also found a polymorphism of the smallest submetacentric chromosomes (B-4) similar to that found by BIANCHI and MOLINA. In order to ascertain the presence of the B-4 chromosomal polymorphism in the rats, we crossed the animals and examined the karyotypes in the hybrids rats. The results of the studies are reported in this paper.

MATERIALS AND METHODS

Rats

Donryu strain rats used in the present study were established by SATO, R. (4, 5) in the Nihon Rat Company. They were obtained at about the 40th generation by brother and sister mating in the company. The other Donryu strain rats purchased in 1961 from the Japan Experimental Animal Research Association, Tokyo were used. These animals have been kept in our laboratory by random-mating, but since 1965 by brother-sister mating.

F-1 hybrid rats: By karyotype study of the Donryu strain rats, the smallest submetaantric chromosomes (B-4) were found to be polymorphic with respect to submetacentrics (type I) and subtelocentrics (type II). Therefore, a male of type I was mated to a female of type II, and *vice versa*. Four litters of F-1 rats were obtained in each crossing and the chromosomes were observed.

For the control groups, a litter of Donryu strain rats (2 males and 4 females) at the 41st generation and another litter (3 males and 3 females) at the 42nd generation were used. The rats used were all at about 10 days old.

Preparation for chromosome study

Chromosmes were obtained from a) bone marrow cells and b) culture cells of the tissue derived from the rat tail.

a) Bone marrow cells: To about 10 days old rats, 30 γ colchicine/rat is injected intraperitoneally one hour prior to sacrifice, animals are killed by dislocation of the cervical vertevrae, femurs and tibias are removed from both legs, both ends of these bones are cut off, saline solution warmed to 37°C is poured into diaphyseal cavity to wash out bone marrow cells, and the cells are collected.

b) Cultured cells: Tail of the rat was cultured *in vitro* by YOSIDA's technique (6) for observation of chromosomes. Namely, the tail of animal is aseptically cut off, the skin with fur is peeled off, then the remaining tail with cartilages and bones is cut into small pieces with ophthalmic scissors, these tissue pieces are thinly spread on the bottom of TD-15 culture bottles, and the tissue culture is carried out in the Eagle's MEM medium supplementing with 20% BS at 37°C. Thereafter the medium is exchanged once or twice, and in about one-week culture fibroblast-like cells are seen to proliferate over the bottom surface of the culture vessel. About 4—5 hours before harvesting, the cells are treated with colchicine adjusted to the concentration of 1γ/ml, and then 0.2% trypsin-PBS solution is added to detach the cells.

The technique employed in the preparation of chromosome specimens in this experiment is essentially the same at that reported by other investigators (7). Namely, the cell suspensions harvested by a) and b) methods are put into the 5ml-centrifugal tubes, centrifuged at 1,500 rpm for 3 minutes to collect the cells, after discarding the supernatant, the cells are suspended in 2 ml of 1% sodium citrate warmed to 37°C, mixed well, and warmed to 37°C for 20 minutes. Next, by addiug 4 drops of the fixative composed of acetic acid: methyl alcohol (1:1, v/v), the mixture is stirred gently and is left standing at room temperature for 10 minutes. Again the cell suspension is poured over with 1 ml fixative and left standing for 20 minutes, centrifuged, after discarding the supernatant, the fixed cells are sedimented and resuspended, during which period fresh fixative is changed 3 times, and finally a small amount (cir. 0.5 ml) of highly condensed cell suspension is obtained. One or two drops of it is put on a clean, wet slide glass previously kept immersed in 50% metanol solution, and dried rapidly over gas-flame. The dried specimens are than stained with 6% Giemsa solution for 3—4 hours, and mounted with balsam.

Sketches and photographs of chromosomes

In order to count and identify individual chromosomes as accurately as possible, those chromosomes in metaphase that are well spread and intact are selected, and sketched by aid of the sketching apparatus of Erma Co. under oil-immersion microscope. More than 50 metaphase chromosomes per sample are sketched, and out of these ten of the most distinct ones are photographed and the karyotypes are determined. For the photography Olympus camera PM-6 and Zeiss's green filter VG-19 are used. The films are "Minicopy" (Fuji), and for the printing, papers of V-4 and V-5 (Gekko) are used, magnification at about × 3,000.

Classification and designation of chromosomes

Classification and designation of chromosomes of normal rat is done according to the method of KURITA *et al* (8). Namely, individual chromosomes are divided into 3 groups of A, B and C according to the position of centromere, (A-group consists of metacentric chromosomes, B-group submeta-subtelocentric chromosomes, and C-group telocentric chromosomes), and further individual chromosomes of each group are arranged in descending order of length and designated by group name and number (i. e., B-1, B-2, …).

RESULTS

Normal karyotype of Donryu strain rats maintained by random mating

Diploid chromosome numbers in 54 Donryu rats (27 males and 27 females) were 42. They were classified into 7 pairs in A-group (metacentrics), 4 pairs in B-group (subtelo-submetacentric) and 10 pairs in C-group (telocentrics) including X and Y chromosomes (Fig. 1).

Table 1 gives the arm ratio (long arm/short arm) and the relative length of chromosomes of the 10 metaphase cells in the bone marrow of one male and one female. It was not possible to identify X-chromosome from its morphological features such as arm ratio and relative length, but for convenience of assaying its chromosome it was allocated to C-4 by referring the Yosida's studuy (7). The Y chromosome, however, was readily distinguishable as the shortest, unpaired one in C-group.

Table 1 Relative length of individual chromosomes taking C-1 as 1.0 and arm ratio (long arm/short arm) in normal diploid complement (10 metaphases).

		A-1	A-2	A-3	A-4	A-5	A-6	A-7
♂	Arm ratio	1.2	1.3	1.2	1.1	1.1	1.1	1.1
	Relative length	0.42	0.39	0.36	0.33	0.31	0.27	0.21
♀	Arm ratio	1.2	1.2	1.1	1.1	1.1	1.1	1.0
	Relative length	0.42	0.39	0.36	0.34	0.31	0.26	0.22

		B-1	B-2	B-3	B-4	
♂	Arm ratio	5.5	2.2	3.3	1.8	3.0
	Relative length	1.11	0.41	0.38	0.28	0.22
♀	Arm ratio	5.7	2.3	4.2	1.9	3.2
	Relative length	1.03	0.41	0.40	0.30	0.23

		C-1	C-2	C-3	C-4(X)	C-5	C-6	C-7	C-8	C-9	C-10	Y
♂	Relative length	1.00	0.72	0.65	0.62	0.58	0.54	0.51	0.48	0.44	0.41	0.30
♀		1.00	0.68	0.63	0.60	0.57	0.53	0.50	0.46	0.43	0.40	

A-group could be divided into two subgroups: i. e. 5 pairs of A-1 to A-5, and two pairs of A-6 and A-7. However, there is no characteristic difference to distinguish individual chromosomes from one another. In B-group it was possible to distinguish each of the four pairs from each. B-1 is the longest subtelocentric. B-2 and B-3 are about the same in size, but B-2, characterized by submetacentric, was distinguished from B-3 in its arm ratio. The short arm of B-3 was dot-like structure, and is rather subtelocentric. B-4, being still shorter than B-2 and B-3, can readily be identified. The chromosomes revealed clearly polymorphic features: namely, a homomorphic pair of submetacentrics (type I), (Fig. 2) homomorphic pair of subtelocentric (type II), (Fig. 3) and heteromorphic pair of the former two chromosomes (type III), (Fig. 1).

C-group was consisted of C-1 to C-10 in which sex chromosomes are included. C-1, being the largest of telocentrics, is readily identified. The length from C-2 to C-10 pairs gradually decreased, but there were no distinct morphological differences among them.

Polymorphism of B-4 chromosomes

Number of rats with types I, II and III of B-4 chromosomes in the Donryu strain rats examined during the period of November, 1967 and May, 1968 in our laboratory is shown in Table 2.

Table 2 Polymorphic types in B-4 chromosome of Donryu strain rat: There are three morphologic types in B-4 chromosomes of the rats. (I): a homomorphic one in which both chromosomes are submetacentric, (II): another homomorphic one of subtelocentric, (III): a heteromorphic one in which one chromosme is submetacentric and the other subtelocentric and this one can be considered as a hybrid of the former two. On 27 litters raised during the period from Novemver, 1967 to May, 1968 in our laboratory, 7 litters showed type I, 19 litters showed type II and animals with type III are found only in one litter.

Type of B 4	(I) homologous submetacentric pair		(II) homologous subtelocentric pair		(III) heteromorphic submeta-subtelo pair	
Sex	♂	♀	♂	♀	♂	♀
No. of rat	7	7	19	19	1	1

Among 54 animals, 14 animals showed type I, 38, type II, and only two, type III.

In order to ascertain the polymorphism in B-4 chromosomes, the animal with type I was crossed to the animal with type II, and obtained 4 litters of F-1 hybrids. They had all type III, heteromorphic pair of B-4 chromosomes.

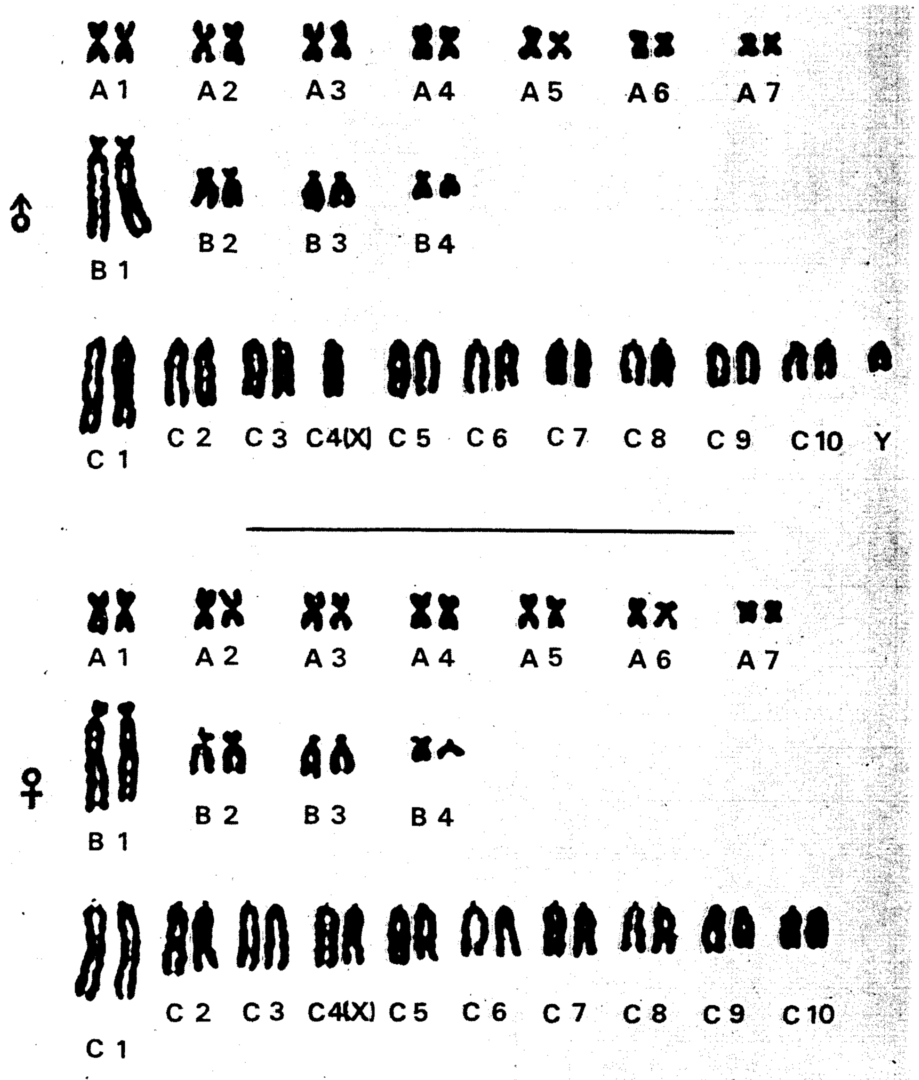

Fig. 1. Normal karyotypes of Donryu strain rat: these metaphases were selected from bone marrow cells of one male and one female with type III of *B*-4 chromosomes respectively. Note that one of B-4 chromosomes submetacentric and other subtelocentric.

Karyotype of inbred Donryu strain rats in Nihon Rat Co.

The karyotype of inbred Donryu strain rats maintained in Nihon Rat Co. was compared with that maintained in our laboratory. Inbred Donryu strain rats were at F-41 and F-42. Karyotypes of inbred Donryu rats in general coincided with that of the animal in our laboratory, showing the

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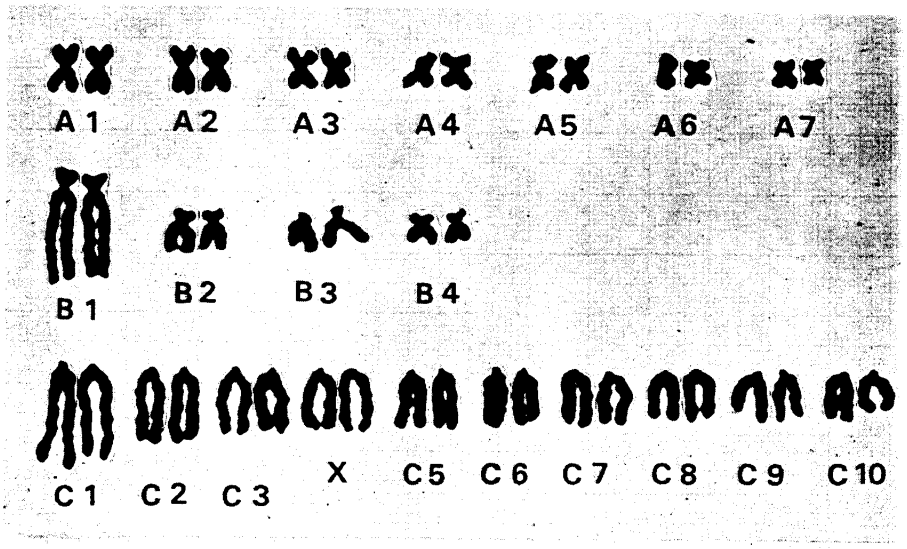


Fig. 2. Karyotype of a bone marrow cell of normal female Donryu strain rat with type I of B-4 chromosomes. Note that B-4 chromosomes are both submetacentric (arrow).

Fig. 3. Karyotype of a bone marrow cell of normal female Donryu strain rat with type II of B-4 chromosomes. Note that B-4 chromosomes are both subtelocentric (arrow).

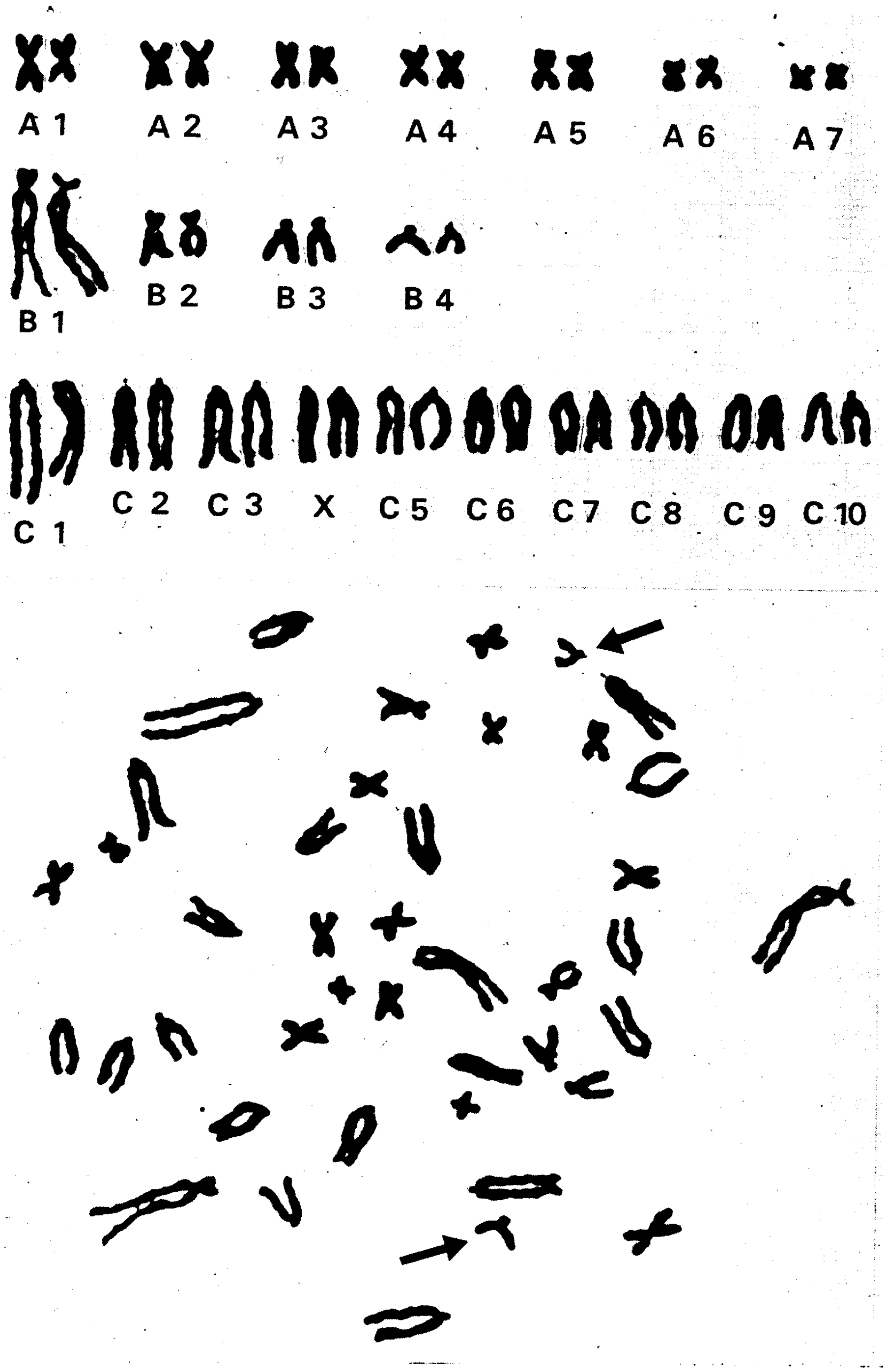
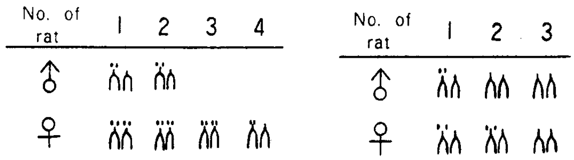

A 1
A 2
A 3
A 4
A 5
A 6
A 7
B 1
B 2
B 3
B 4
C 1
C 2
C 3
X
C 5
C 6
C 7
C 8
C 9
C 10

Table 3 Results of the B-4 chromosomes in inbred Donryu strain rats: a litter of F-41 (2 males and 4 females) showed types I and III and segregated 1 : 1. A litter of F-42 (3 males and 3 females) showed types II and III and segregated 1 : 1.



polymorphism of B-4 (Table 3).

Animals at F-41, irrespective of sex, showed types I and III, but no type II. The ratio of I and III was at 1 : 1, suggesting their parents to be of types I and III. In the case of F-42 there could be observed only types II and III but no type I, of which the segregation of II and III is 1 : 1, indicating their parents could be of types II or III.

DISCUSSION

YOSIDA *et al.* (2) have simply reported about the karyotype of Donryu strain rats. In the present experiment we observed accurately individual chromosomes of normal karyotype in the rats. Findings on the karyotype were essentially the same as those of the former, but the presence of polymorphism in B-4 chromosomes was observed in the present study.

HUNGERFORD and NOWELL (1) have numbered the rat chromosomes in descending order of length and further classified 5 groups according to the shape. Such a classification seems to be extensively used recently. YOSIDA *et al.* (2) have numbered chromosomes in descending order of length and further divided them into 6 groups (A to F) in order of their size and shape. More recently, KIRITA *et al.* (8) have similarly classified chromosomes into 3 groups of A, B and C according to the position of centromere. We have followed the classification by them in the present report.

HUNGERFORD and NOWELL (1) consider that the sex chromosome of rats reveals different morphology according to strains, i. e. it is readily identified in some strains while it is very difficult or completely impossible in others. According to them the X chromosome which was readily distinguishable from the others was called as X_{st}, and that was difficult of identify as X_t. The sex chromosome in Donryu strain rat belonged to the latter type. YOSIDA and AMANO (2) placed the X in the size between the fourth and the fifth pair. REES *et al.* (9) considered that the X chromosome seems to be the second largest terminal chromosomes, but HUNGERFORD and NOWELL (1) placed it between the third pair and the fourth pair. In any case, precise details have to await further investigations.

YOSIDA and AMANO (2) reported about the polymorphism of No. 3 (C-2) autosomes of laboratory breeding rats including Donryu strain and wild rats, but they did not make any mention on polymorphism in B-4. On the other hand, BIANCHI and MOLINA (3) found the polymorphism of B-4 in *Rattus norvegicus*. Namely, among 40 rats, 20 showed both isologous chromosomes to be submetacentric, 12 animals revealed one submetacentric and the other telocentric, and 8 animals, both telocentric. In addition, REES *et al.* (9) compaired the karyotype of several rat strains, and showed that B-4 of most strain rat had satellite, but occasionally heteromorphic one. HUNGERFORD and NOWELL (1) studied the karyotype of three inbred rat strains and found some B-4 pairs had satellites in both, and some pair had satellites in only one of the pair. NOWELL *et al.* (10) claim to have found satellites of B-4 more predominantly in ACI strain than in Buffalo strain. As is obvious from the above reports the presence of satellites in B-4 pair is variegated according to the rat strains.

The polymorphism of B-4 chromosomes observed by the above investigators was confirmed by us in Donryu strain. In the case of Donryu strain rat the major portion of short arm is constituted of satellites, and there are some pairs (type I) that have satellites in both of the pairs, some (type II) that have none, and some (type III) that can be considered as a hybrid of I and II. As has been assumed by BIANCHI and MOLINA (3), we have been able to demonstrate experimentally that type III is a hybrid of I and II.

The karyotype of Donryu strain rats maintained in our laboratory coincided roughly with those maintained at Nihon Rat Co., and the polymorphism was also found similarly in the B-4 chromosome of both rats. However, type III could be found only in two of our rats, and types I and II were predominant in the rest of rats. This fact may be attributable to the fact that we raised these animals mainly by brother-sister mating for the period of six years (1961—1967) so that the majority of the B-4 chromosomes had turned to homologous type. At the present we are trying to raise and maintain the animals of types I and II by keeping them in separate cages. On the other hand, in our study on two litters each unrelated substrain of inbred rats from Nihon Rat Co. B-4 in one litter at F-41 generration showed I and III in the ratio of 1 : 1, and in the other litter of F-42 generation, types II and III were similarly in the ratio of 1 : 1. This is difficult problem to understand because these animals have been maintained over 40 generations by brother-sister mating. It seems that, when the animals are maintained over 40 generations through brother-sister mating, alleromorph would be mostly transformed to homologous type, and likewise even B-4 would naturally turn predominantly to homologous types such as I and II. This problem requires more precise investigations.

* Note: To every individual chromosomes of rats referred to in the text the author's own classification and designations have been given. Therefore, chromosome 13 of Hungerford and Nowell classification is equivalent to B-4 of the author's.

SUMMARY

1) Normal karyotype of Donryu strain rat was determined according to the classification of KURITA *et al.* (8). Namely, the number of chromosomes was 42 in diploid cells, and chromosomes were divided into 3 groups (A, B and C) according to the position of centromere. A-group was consisted of 7 pairs of metacentric chromosomes, B-group 4 pairs of submeta-subtelocentrics and C-group 10 pairs of telocentrics and Y.

2) Among all chromosome pairs a pair of the longest telocentric chromosomes (C-1), 4 pairs of all the B-group, and the Y chromosome were recognizable.

3) The presence of polymorphism was demonstrated in the smallest submetacentric chromosomes (B-4), : namely, (I) a homologous submetacentric pair, (II) a homologous subtelocentric pair and (III) a heteromorphic submeta and subtelocentric pair which seemed to be a hybrid from (I) and (II). To distinguish the polymorphism in their genotype from phenotype was impossible.

4) Animals with type III B-4 chromosomes were produced by type I and type II animals.

5) By checking the chromosomes of the inbred Donryu strain rats maintained over 40 generations by brother-sister mating at Nihon Rat Co., polymorphism in B-4 chromosomes was also recognized.

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